NITRIC OXIDE-RELEASING 1-[(2-CARBOXYLATO)PYRROLIDIN-1-YL] DIAZEN-1-IUM-1,2-DIOLATES AND COMPOSITION COMPRISING SAME

RELATED APPLICATION

This is a continuation of U.S. App. Ser. No. 08/837,812, filed Apr. 22, 1997, which is a divisional of U.S. App. Ser. No. 08/344,157, filed on Nov. 22, 1994, now U.S. Pat. No. 5,632,981, which is a continuation-in-part of U.S. App. Ser. No. 08/121,169, filed on Sep. 14, 1993, now U.S. Pat. No. 5,525,357, which is a continuation-in-part of U.S. App. Ser. No. 07/935,565, filed on Aug. 24, 1992, now U.S. Pat. No. 5,405,919. The entire disclosure of the '812 application and the '981, '357 and '919 patents are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to polymeric compositions capable of releasing nitric oxide. In particular, the present invention relates to polymeric compositions comprising a biopolymer, such as a peptide, polypeptide, protein, oligonucleotide, nucleic acid, or the like to which is bound a nitric oxide-releasing $N_2O_2^-$ functional group, pharmaceutical compositions comprising such polymeric compositions, and methods of treating biological disorders with such a biopolymeric composition.

BACKGROUND OF THE INVENTION

Nitric oxide (NO) has recently been implicated in a variety of bioregulatory processes, including normal physiological control of blood pressure, macrophage-induced cytostasis and cytotoxicity, and neurotransmission (Moncada et al., "Nitric Oxide from L-Arginine: A Bioregulatory System," *Excerpta Medica*, International Congress Series 897 (Elsevier Science Publishers B. V.: Amsterdam, 1990); marletta et al., "Unraveling the Biological Significance of Nitric Oxide," *Biofactors*, 2, 219–225 (1990); Ignarro, "Nitric Oxide. A Novel Signal Transduction Mechanism for Transcellular Communication," *Hypertension* (*Dallas*), 16, 477–483 (1990)). A number of compounds have been developed which are capable of delivering nitric oxide, including compounds which release nitric oxide upon being metabolized and compounds which release nitric oxide spontaneously in aqueous solution.

Those compounds which release nitric oxide upon being metabolized include the widely used nitrovasodilators glyceryl trinitrate and sodium nitroprusside (Ignarro et al., *J. Pharmacol. Exp. Ther.*, 21, 739–749 (1981); Ignarro, *Annu. Rev. Pharmacol. Toxicol.*, 30, 535–560 (1990); Kruszyna et al., *Toxicol. Appl. Pharmacol.*, 91, 429–438 (1987); Wilcox et al., *Chem. Res. Toxicol.*, 3, 71–76 (1990). Another compound, S-nitroso-N-acetylpenicillamine, has been reported to release nitric oxide in solution and to be effective at inhibiting DNA synthesis (Garg et al., *Biochem. and Biophys. Res. Comm.*, 171, 474–479 (1990)).

Numerous nitric oxide-nucleophile complexes have been described, e.g., Drago, *ACS Adv. Chem. Ser.*, 36, 143–149 (1962). See also Longhi and Drago, *Inorg. Chem.*, 2, 85 (1963). Some of these complexes are known to evolve nitric oxide on heating or hydrolysis, e.g., Maragos et al., *J. Med. Chem.* 34, 3242–3247 (1991).

The cytostatic effect of nitric oxide solutions on tumor cells in vitro has been demonstrated. In particular, it has been shown that solutions of nitric oxide inhibit DNA synthesis and mitochondrial respiration of tumor cells in vitro (Hibbs et al., *Biochem. and Biophys. Res. Comm.*, 157, 87–94 (1988); Stuehr et al., *J. Exp. Med.*, 169, 1543–1555 (1989)).

Endothelium-derived relaxing factor (EDRF) is a labile humoral agent which is part of a cascade of interacting agents involved in the relaxation of vascular smooth muscle. EDRF is thus important in the control of vascular resistance to blood flow and in the control of blood pressure. Some vasodilators act by causing EDRF to be released from endothelial cells. (See Furchgott, *Ann. Rev. Pharmacol. Toxicol.*, 24, 175–197 (1984).) In 1987, Palmer et al., presented evidence that EDRF is identical to the simple molecule, nitric oxide, No (*Nature*, 317, 524–526 (1987)), though more recently, that conclusion has been challenged (Myers et al., *Nature*, 345, 161–163, 1990)).

Nitric oxide in its pure form, however, is a highly reactive gas having limited solubility in aqueous media (WHO Task Group on Environmental Health Criteria for Oxides of Nitrogen, *Oxides of Nitrogen*, Environmental Health Criteria 4 (World Health Organization: Geneva, 1977)). Nitric oxide, therefore, is difficult to introduce reliably into most biological systems without premature decomposition.

The difficulty in administering nitric oxide can be overcome in some cases by administering nitric oxide pharmacologically in prodrug form. The compounds glyceryl trinitrate and sodium nitroprusside are relatively stable and release nitric oxide only on activation (Ignarro et al., *J. Pharmacol. Exp. Ther.*, 218, 739–749 (1981); Ignarro, *Annu. Rev. Pharmacol. Toxicol.*, 30, 535–560 (1990); Kruszyna et al., *Toxicol. App. Pharmacol.*, 91, 429–438 (1987); Wilcox et al., *Chem. Res. Toxicol.*, 3, 71–76 (1990)). While this feature may be an advantage in some applications, it can also be a significant liability, as in the development of tolerance to glyceryl trinitrate via the exhaustion of the relevant enzyme/cofactor system (Ignarro et al., *Annu. Rev. Pharmacol. Toxicol.*, 25, 171–191 (1985); Kuhn et al., *J. Cardiovasc. Pharmacol.*, 14 (Suppl. 11), S47-S54 (1989)) and toxicity from metabolically produced cyanide during prolonged administration of nitroprusside (Smith et al., "A Potpourri of Biologically Reactive Intermediates" in *Biological Reactive Intermediates IV. Molecular and Cellular Effects and Their Impact on Human Health* (Witmer et al., eds.), Advances in Experimental Medicine and Biology Volume 283 (Plenum Press: New York, 1991), pp. 365–369).

Evidence that nitric oxide is released from the endothelial cells and is responsible for the relaxation of the vascular smooth muscle, and hence the control of blood pressure, has resulted in the development of artificial agents that can deliver nitric oxide in vivo. A very important class of such agents is the nitric oxide-nucleophile complexes. Recently, a method for treating cardiovascular disorders in a mammal with certain nitric oxide-nucleophile complexes was disclosed, e.g. in U.S. Pat. No. 4,954,526. These compounds contain the anionic $N_2O_2^-$ group or derivatives thereof. See also, Maragos et al., *J. Med. Chem.*, 34, 3242–3247 (1991). Many of these compounds have proven especially promising pharmacologically because, unlike nitrovasodilators such as nitroprusside and nitroglycerin, they release nitric oxide without first having to be activated. The only other series of drugs currently known to be capable of releasing nitric oxide purely spontaneously is the S-nitrosothiol series, compounds of structure R-S-NO (Stamler et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89, 444–448 (1992); Stamler et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89, 8087–8091 (1992)); however, the R—S—NO—NO reaction is kinetically complicated and difficult to control (Morley et al., *J. Cardiovasc. Pharmacol.*, 21,